

Figure

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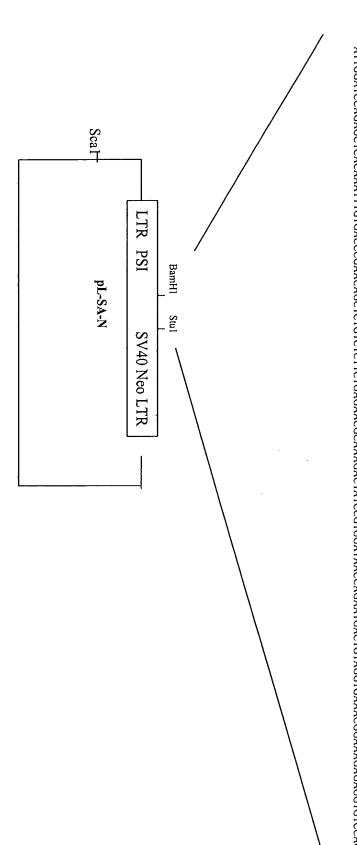
Bbe1 overhang Splice donor (underlined) Start of MLV R (in italics) Kpn1 overhang

U3 R U5
Bbel Kpnl
pLTR



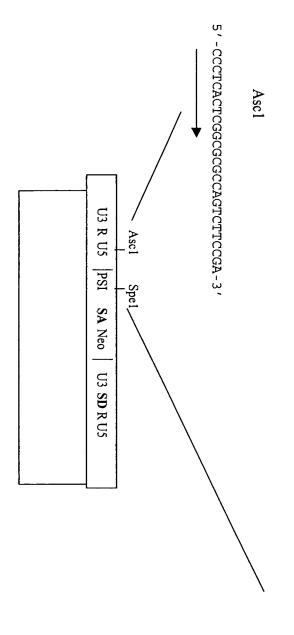
Figure 3







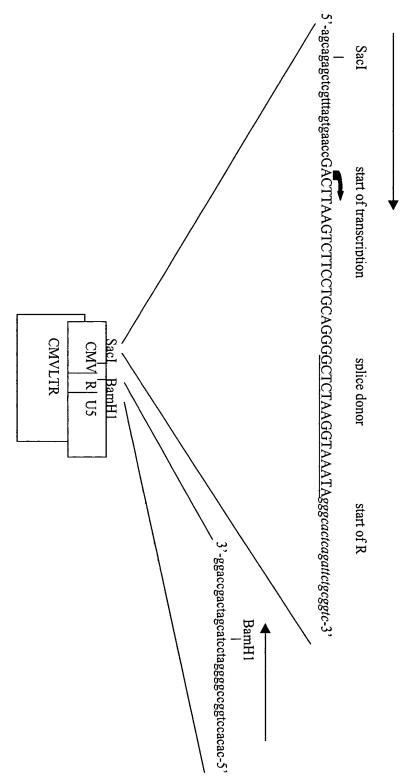
GT TO GC CHANGE Spe1



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GAGCCAAAGCCGCGGCCCTTCCGTTTCTTTGCTTTTGAAAGACCCCACCCGTAGGTGGCAA





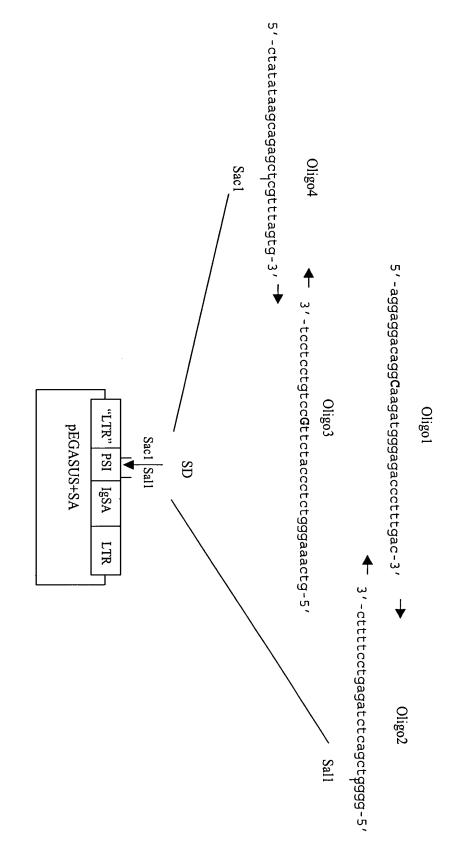


overhang Xho1 Branch point

5'-TCGACGTTTAAACACTGGGCTTGTCGAGACAGAGAAGACTCTTGCGTTTCTGATAGGCACCTATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTCACGTGAAGCTAGCCTCGAGTTGGC-3' SA Bpu1102 overhang

 ${\tt GCAAATTTGTGACCCGAACAGCTCTGTCTTGTGAGAACGCAAAGACTATCCGTGGATAACCAGAATGACTGTAGGTGAAACGGAAAGAGAGGTGTCCAGTGCACTTCGATCGGAGCTCAACCGACT$ "LTR" PSI pEGASUS-1 XhoI CMVlacZ Bpu1102 LTR







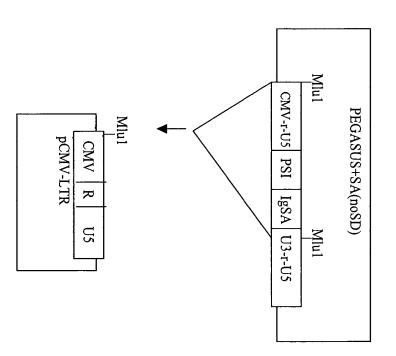


Figure 9



Figure 10

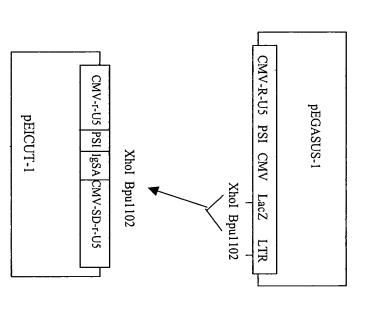


Figure 11A

pEICUT-LacZ sequence

GCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAAGCCAATATTGAAACCCACGGCATGGTGCCAATGAATCGTCTGACCGATGATCCGCGCTGGCTACCGGCGATGAGCGCGAACGCGTAACGCGAATGGTGCAGCGCGATCGTA GCTGGGTCGGTTACGGCCAGGACAGTCGTTTGCCGTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGGAGAAAACCGCCTCGCGGTGATGGTGCTTGCGTTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGAT AAGGGTCTCAGAAATTAACTACTGGTAACTGTAATTGGGCGCTAAGTCTAGTAGACTTATTTCATGATACCAACTTTGTAAAAGAAAAAGGACTCTAGAGTCGACCCCCTCGACGTTTAAACACTGGGCCTTGTCGAGACAGAGAAGA CGTCAATGGGTGGAGTATTTACGGGTAAACTGCCCACTTGGCAGTACATGAGTGTATCATATGCCCAAGTCCGCCCCCTATTGACGTCAATGACGGTAAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTACGGGACTTTCC ATGTCGCCGATGTGAGTTTCTGTGATAACTGATATCGCCATTTTTCCAAAAGTGATTTTTGGGCATACGCGATATCTGGCGATAGCGCTTATATCGTTTACGGGGGATAGACGACTTTGGTGACTTTGGGCGATTCTGTGTC GCCATTTTTCCAAAAGTGATTTTTGGGCATACGCGATATCTGGCGATAGCGCTTATATCGTTTACGGGGGATGGCGATAGACGACTTTGGTGACTTGGGCGATTCTGTGTGTCGCCAAATATCGCAGTTTCGATATAGGTGACAGA GAATATCGACGGTTTCCATATGGGGATTGGTGGCGACGACTCCTGGAGCCCGTCAGTATCGGCGGAATTCCAGCTGAGCGCCGGTCGCTATACCAGTTGGTCTTGGTGTCAAAAATAATAATAATAACCGGGCAGGGGGGGATCCGC AGCTGGCGCAGGTAGCAGAGCGGGTAAACTGGCTCGGATTAGGGCCGCAAGAAAACTATCCCGACCGCCTTACTGCCGCTTTTTGACCGCTTGGCGATTTGTCAGACATGTATACCCCCGTACGTCTTCCCGAAGAAAAC GATAACGACATTGGCGTAAGTGAAGCGACCCGCATTGACCCTAACGCCTGGGTCGAACGCTGGAACGCTGGACCGCGCGCCGTTACCAGGCCGAAGCAGCGTTGTTGCAGTGCACGGCAGATACACTTGCTGATGCGGTGCTGATTACGAC ATTATTTGCCCGATGTACGCGCGCGTGGATGAAGACCAGCCCTTCCCGGCTGTGCCGAAATGGTCCATCAAAAAATGGCTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGTAACAGTCT CCGAAATCTCTGATCGTGCGGTGGTTGAACTGCACACCGCCGACGCTGATTGAAGCAGAAGCCTGCGATGTCGGTTTCCGCGAGGTGCGGATTGAAAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATT AGCGGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCGACTACACAAATTCAGCGATTTCCATGTTGCCACTTCGCTTTAATGATGATTTCAGCCGCGCTGTACTGGAGGCTGAAGTTCAGATGTGCGGGGAGTTGCGTGACTACCTGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTAACTCGGCGTTTCATCTGTGGTGCAACGGGCCTGATCGTAGGATCCCCGGGACAGCAGAGGAGAACTTACAGAAGTCTTCTGGAGGTGTTCCTGGCCAGAACACAGGAGGACAGGTAAGATGGGAGACCCTTTGACATGGAGCAAGGCGCTCAAGAAGTTAGAGAAGTGACGGTAC ACCAAAATCAACGGACTTTCCAAAATGTCGTAACAACTGCGATCGCCCCCCGTTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGGGCACTCAGATTCTGCGGTCTGA TGAATAATAAAATGTGTGTTTTGTCCGAAATACGCGTTTTGAGATTTCTGTCGCCGAAATATGTCATGTCGCGCGATAGTGGTGTTTTATCGCCGATAGAGATGGCGATATTGGAAAAATTTGAAAATATGGCATATTTGAAA ${\tt CATACGTTGTATCCATATCGTAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTATTGACTA}$ CGATATGAGGCTATATCGCCGATAGAGGCGACATCAAGCTGGCACATGGCTGTCTATCGATCTATACATTGAATCAATATTGGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCCATTGGCCATT TTTTGAGATTTCTGTCGCCGACTAAATTCATGTCGCGCGATAGTGGTGTTTATCGCCGATAGAGATGGCGATATTGGAAAATTTGAAAATATTGGCATATTGAAAATGTCGCCGATGTGAGTTTCTGTGTAACTGATATC



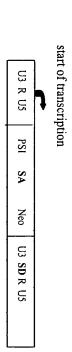
Figure 11B

AACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCG AAACAAAAAACCACGGTAGCAGGGGTGGTTTGTTTGCCGGATCAAGAGGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACA ${\tt CGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCT$ AGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTTCGCCCCGAAGAACGTTTTCCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCG $\tt ATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTTGCTCACCCAGAAACGCTGGTGAA$ GCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTTCTAAATACATTCAAATATGTATCCGCTC ACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGGCTTACAGACAAGCTGTGACCGTCTCCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGACGCAAAGG ${ t GCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTGATCATCGTGAAGAGCTTGGCGGAATGGGC$ AAGATGGATTGCAGGTGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTTGTCAAC CAGAGGCCGAGGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTGATTCTTCTGACACAACAGTCTCGAACTTAAGGCTAGAGCCACCATGATTGAAC CCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCCCCTATTGACGTCAATGACGGTAAATGGCCCG ACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGGCTCGACAGATCT TTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCGGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTG





(A) pICUT vector in transfected cells



(B) pICUT vector in transduced cells

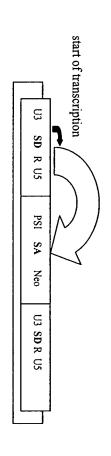
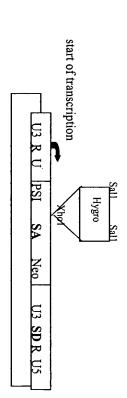




Figure 13

(A) Vector configuration in transfected cells



(B) Vector configuration in transduced cells

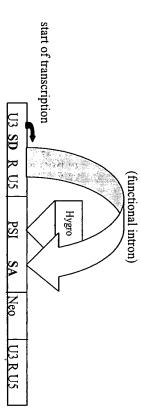
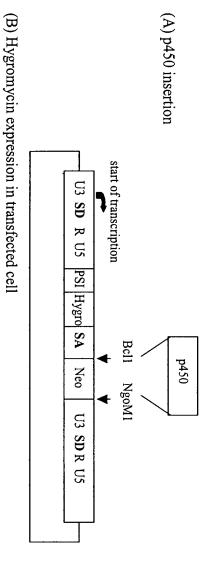




Figure 14

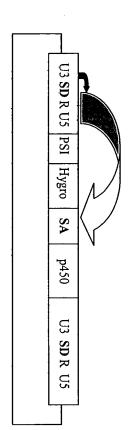


start of transcription

U3 R U5 PSI Hy

U3 R U5 PSI Hygro SA p450 U3 SD R U5

(C) p450 expression in transduced cell





3'end of pol 5'altered 4070A 5'-ATG CGT TCA ACG CTC TCA AAA CCC CTT AAA AAT AAG 5'-ATG GCC AGA AGC ACC CTG AGC AAG CCA CCC CAG GAC

GTT AAC CCG CGA GGC CCC CTA ATC CCC-3' AAA AAT CCC TGG AAA CCT CTG ATC GTC-3'



GAAGAATGIT GITTITATGO AGAGAAAGGC TTAATCAGAG CTGTTTAATA GATCCCCCTG GTACTCTTAC TGATCTTACT AAAGACAGGA TCTCAGTGGT CCCATAGAGT ACGAGCCATG		4- 4- 1- 1- 1-	
TATEC CAGAG CCCTG TTACT GTGGT CCATG	CTCTG ACCAA ATGGG TTTGA TACCT AACCT GATTT	CAGCC CTTAA CCTTA AACTG TGCAT TCAGG	CTGAG GCCAA CTATG GGCTG GGGCA TGTGA AAGCG GACCT CTAGT GGACT CTCCC AGTTA
AGACCACACG ACAAAAACTA GTTTACCACC CTTTGGACCT CCAGGCTCTG	GCCCAGAGTA ATATAAAAGA AGGGATTGCA AGGGACTTCAT AGAAAAGTCA GCTATTCCTA GCTATTCCTA	ACCCCCAGGA CCTCACCAAT TTACGAAGGA TACGGCCACT GGGGGCAGTA ATCCTACTAC CTGCTTGTCC	CAAGCCACCC AGGAGTAGGG CACCAACCTG TGCCACCTCC TGATCTGGTC CAAGTACCCC TACCGTAAAG AACCACCACCC CTCCAAACTA CGGTAACTA CCTCAAAATTC GAGACTGTAC TAATGTGGGA TTCCTCACCA TTCCTCACCA CCCCCTTCACCA TTCCTCACCA CCCCCCTTCACCA CCCCCCTTCACCA CCCCCCTTCACCA CCCCCCTTCACCA CCCCCCTTCACCA CCCCCCCTTCACCA CCCCCCCTTCACCA CCCCCCCTTCACCA CCCCCCCTTCACCA CCCCCCCTTCACCA CCCCCCCTTCACCA CCCCCCCTTCACCA CCCCCCCTTCC
GGGCTAGTGA TTTGAGACAG TTAATCTCCA TGCATTCTCA GTTTTGACTC	ATTTACCACT GAGCCAGTAT GCTGGAATAG GCCGCTATCC CTGACCTCGT AAGGAGGGAG AAGGAGGGAG	ACTGGAGATA CCCGACAAGA GTAGCGGTCG TCCCAACATA CCTAAAAACTC CTTGCAGCAC ACCACGGTGC	CAGGACAAAA ATGGCAGAGA CTCCTGGGAA GGAGAGGAGTTGCAGGGTTACT CCCTGGGGACA TCCAATTCCT ACTGATGCAG CCGGACAGGTTC ACTGATGCAGATTC ACTGAGAGATTC ACTGAGAGATTC ACTGAGAGATTC ACTGAGAGATTC ACTGAGAGATTC ACTGAGAGATTA ACTGAGAGATTA
GAGACAGCAT GCCAAGGATG CCATCATGGG ATCGATTGGT AGCAATATC (CCCCCGATTA CATTGACCCT GGACGGGGAC AGACAGACCT TGTCTGAAGT GTCTCTGCGC CACCCCCCCCCC	GACTACTAGC CCCAAGAATG TGGGCACTTA AGCTTACCCT ACCAGGCCTT CCGCCGGAAC TCAATCTAAC	TCAATCCCTG GCCCCATC CTGTACAAGA GGGACCCTTC AGCGGACCAGG GGGAAGCCAC CCGGGATGCTC TCCAGAGGGC CAGATCCTAT CCATAGGGC TACCGGCTCC TACCGGCTCC TACCGGCTCC TACCGGCTCC TACCGGCTCC TACCGGCTCC TACCGCCTCAAC
GGCCAAATTA GTTCGAAGGG ACCTCTAATA CCAATTTGTT CCAGCTAAAA	TATGTATGGT GGCCCTTCTA CACTGCCTTA CAACGAAGTC AGTCCTACAG AGCCCTAAAA	TCTAGTCAAA TTGGCTGTGC TACCAATCAT ATCTGAAGTG ATGTAACACC AATGTGGGCT CACAGATTAT	GAAACCTCTG AGGTC TGCCTTCCCA AGACCAGGAA GACTTTTGAC AGAGGGCTAC ATCATCGTGG TAAAGTTGCC TACTCGAGGG TAACTGGAGG TAACTGGAGG TAACTGGAGG TAACTGGAGG TAACTGGACG TACCCAGTA ACAGCCACTTC CCAACCCAGTA ACAGCCACTACA TGCCCCTACA



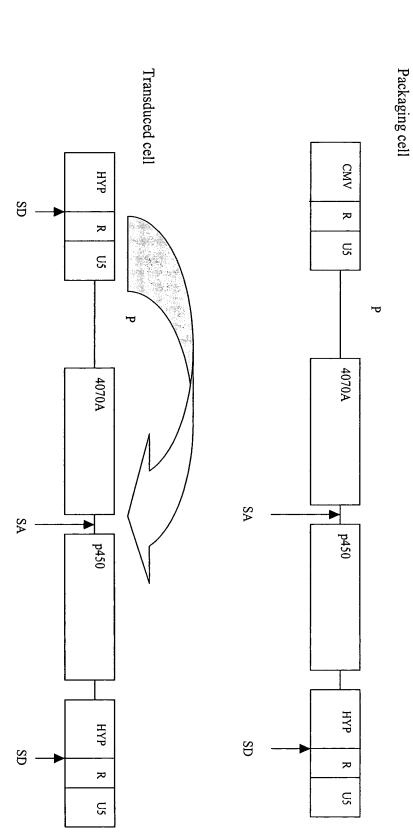
HYP= Hypoxia responsive promoter
P= MLV packaging signal
4070A= MLV amphotrophic Env gene
p450= p450 reducatase encoding cDNA

CMV= CMV Promoter

SD= Splice donor

SA= Splice acceptor

Figure 17





U3

5'-p450

R

US

U3

5'-p450

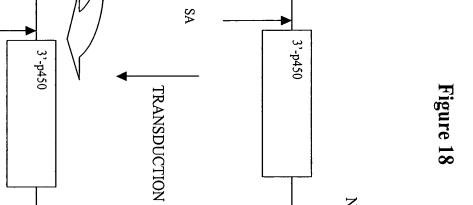
R

U5

SD

SA

SD



SD

CMV

R

US

Nhel

UЗ

5'-p450

R

US

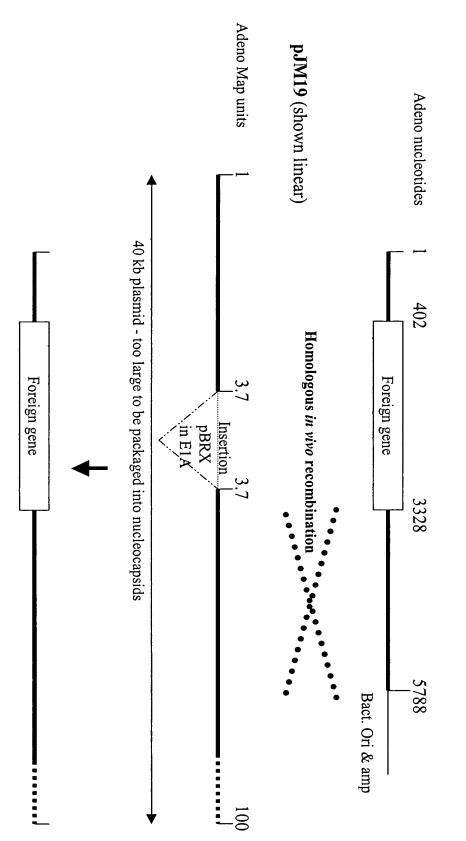


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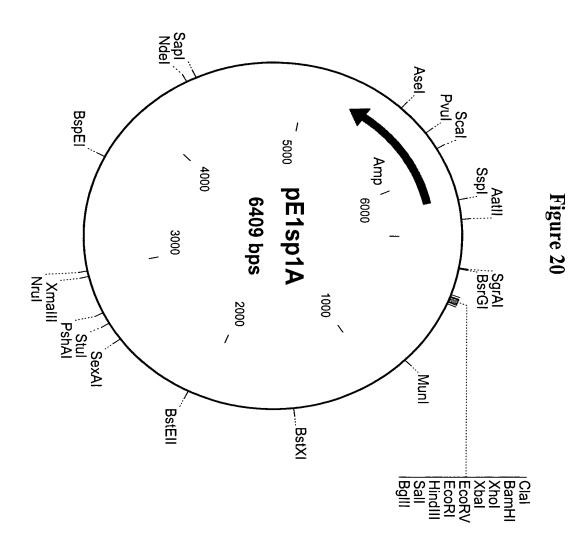
Figure 19

Transfer vector (shown linear)

or of









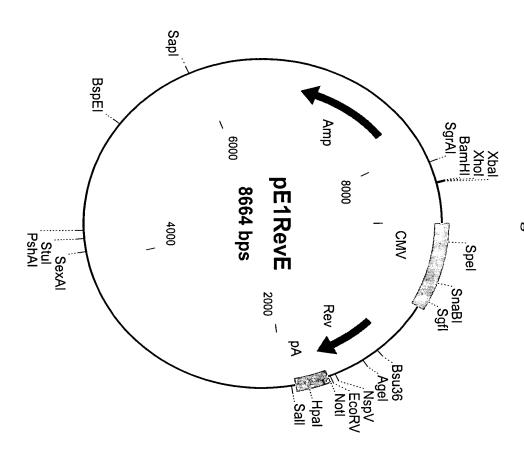


Figure 21



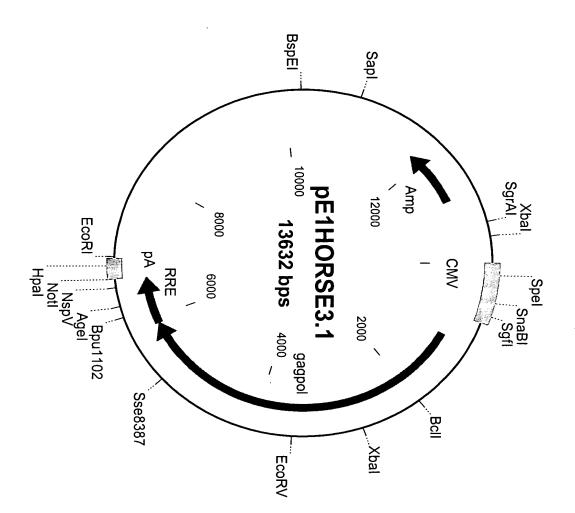
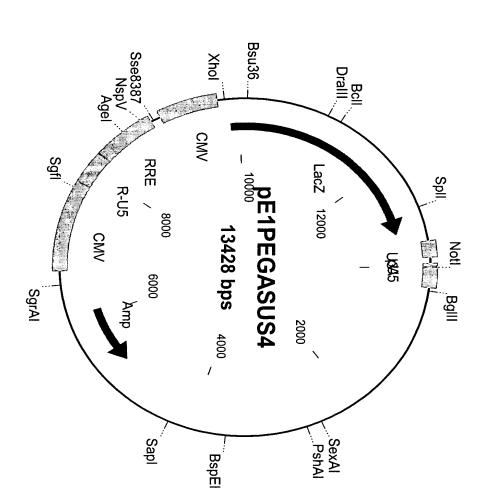


Figure 22









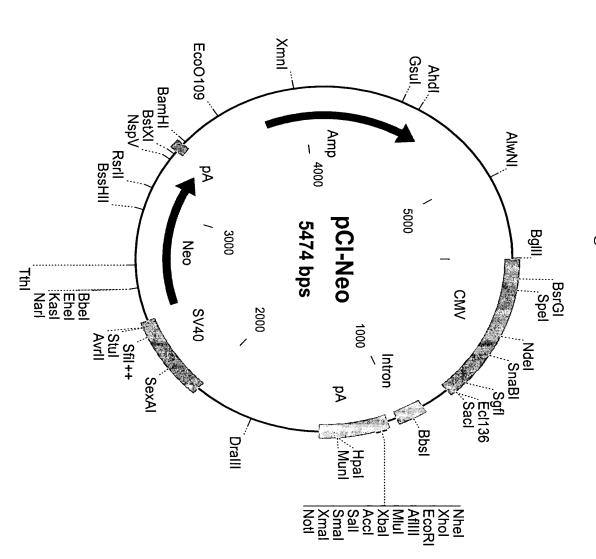


Figure 24

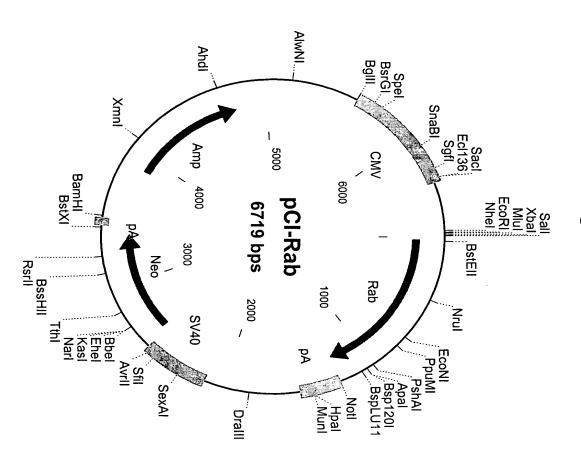


Figure 25

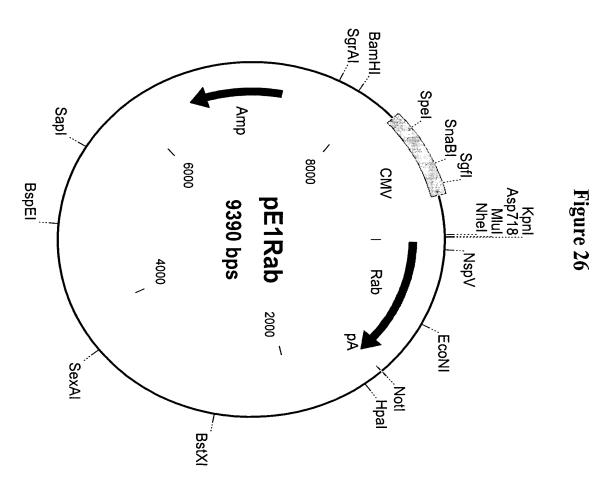
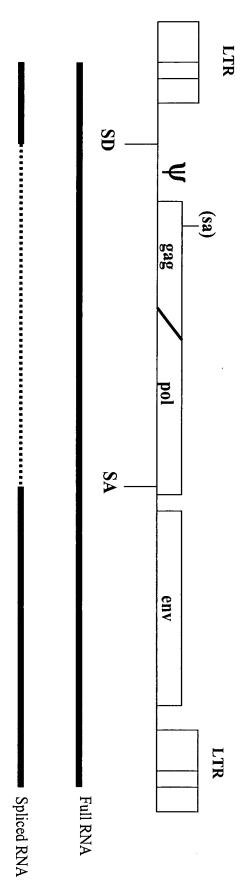


Figure 27a

A) Natural splicing configuration

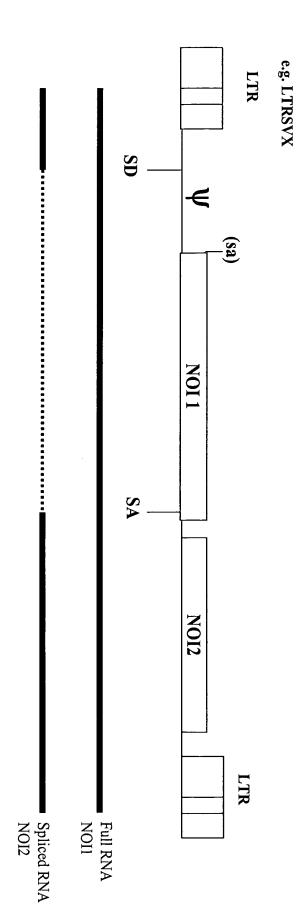


L 1 3

SD = Splice donor
SA = Splice acceptor
(sa) = cryptic splice acceptor
ψ = packaging site

Figure 27b

Splicing configurations in known vectors



SD = Splice donor SA = Splice acceptor

(sa) = Cryptic splice acceptor

 ψ = packaging site

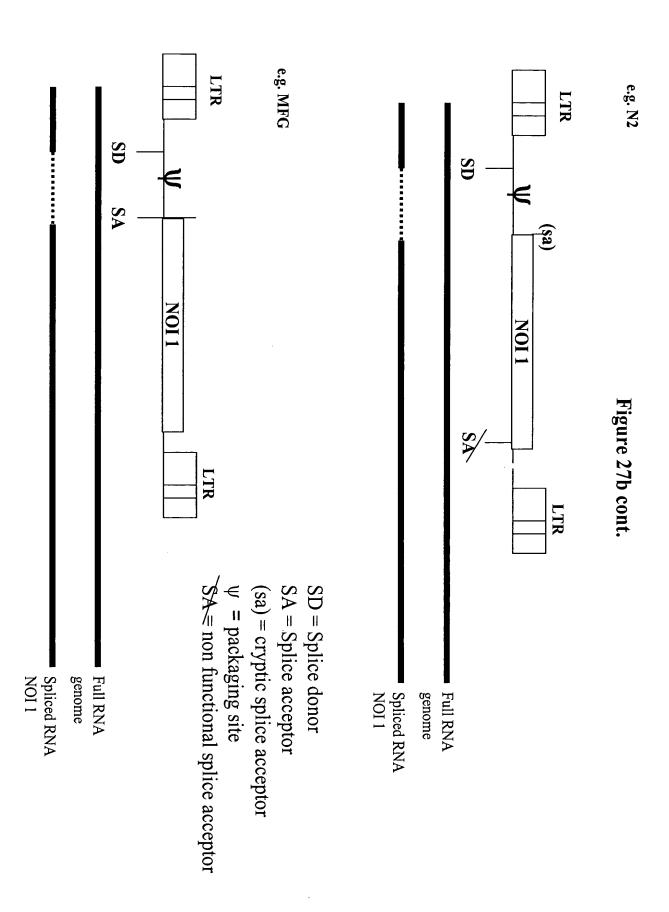
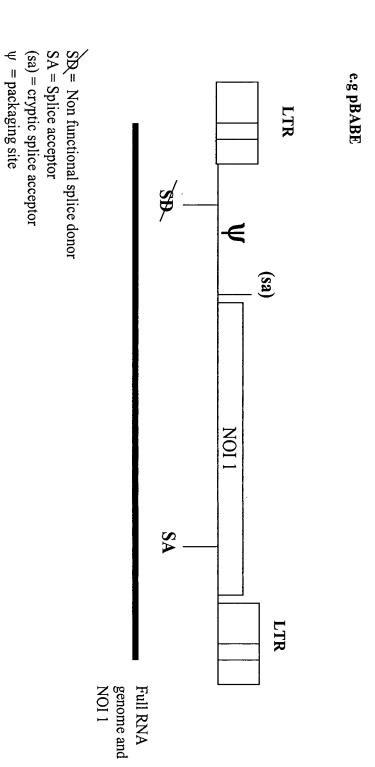
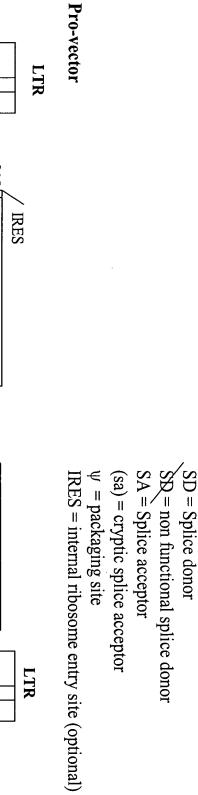


Figure 27b cont.



Full RNA

Figure 27c



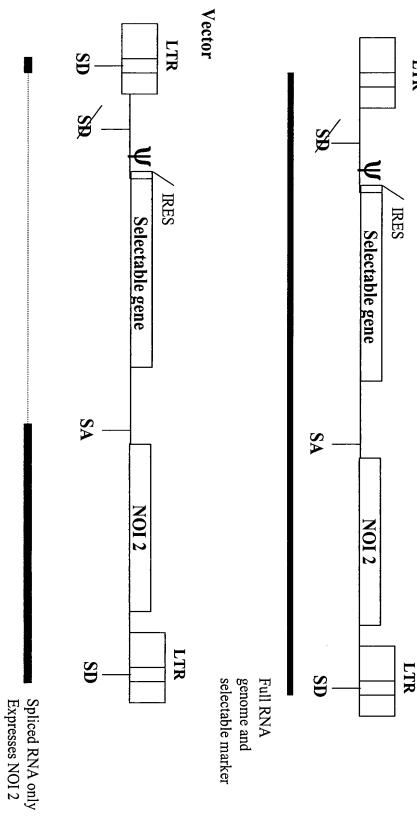


Figure 27c cont.

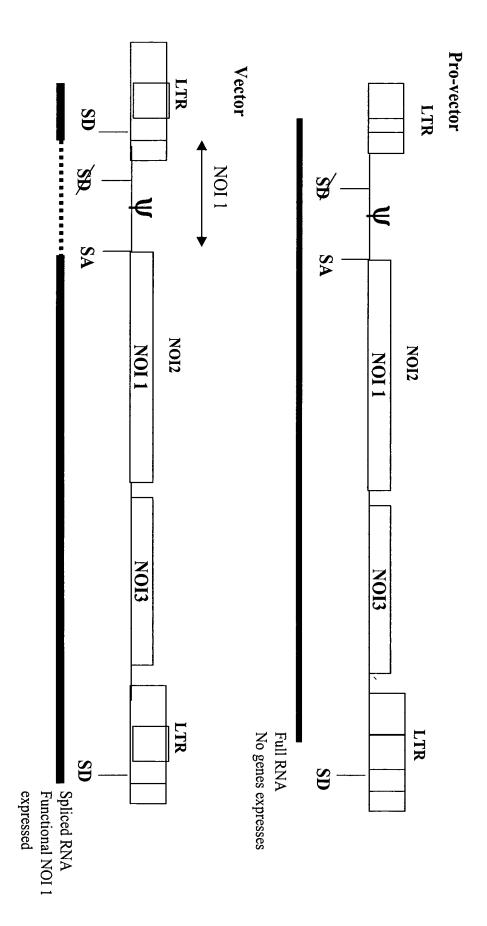
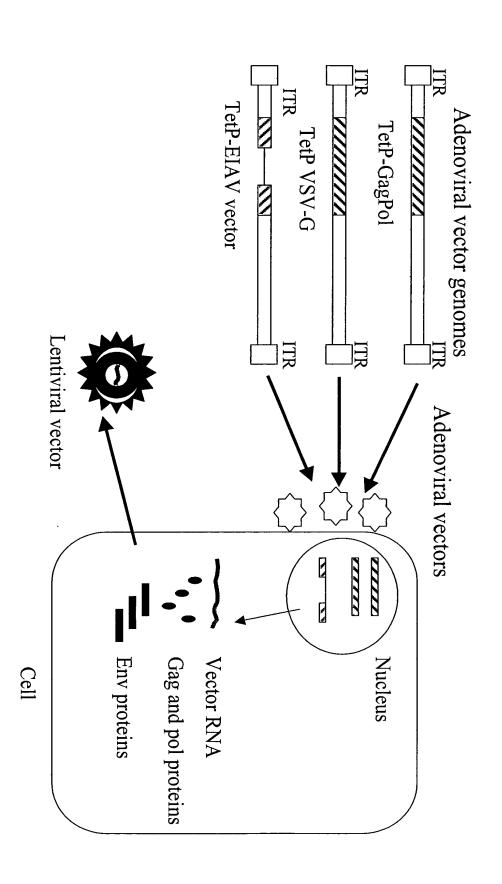


Figure 28



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